

## Four spontaneous pregnancies and three live births following subcutaneous transplantation of frozen banked ovarian tissue: What is the explanation?

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**Objective:** To report the long-term follow-up of an experimental heterotopic ovarian transplantation with frozen-thawed ovarian tissue.

**Design:** Long-term follow-up of an experimental surgery; case report.

**Setting:** Academic reproductive medicine center.

**Patient(s):** A 28-year-old cancer survivor with previous Hodgkin disease and relapse.

**Intervention(s):** Laparoscopic oophorectomy for ovarian cryopreservation before preconditioning chemotherapy for hematologic stem cell transplantation. Ovarian tissue thawing and subcutaneous heterotopic ovarian transplantation in the lower abdominal wall 2½ years after the hematologic stem cell transplantation.

**Main Outcome Measure(s):** Resumption of ovarian function after transplantation, recovery of fertility, and pregnancy outcome.

**Result(s):** Follicle development was observed in the graft 2 months after transplantation, and a *P* value of 14 ng/mL indicated ovulation. The patient conceived spontaneously four times within 5 years and delivered three children. The in situ ovary remained atrophic but showed occasional follicle activity contemporaneously with the graft.

**Conclusion(s):** The mechanism behind spontaneous restoration of fertility with consecutive viable pregnancies after a heterotopic ovarian transplantation needs to be explored. Further laboratory and clinical research will be needed to explore the true origin of pregnancies after ovarian transplantations. (*Fertil Steril*® 2011;95:804.e7–e10. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Fertility preservation, cancer, Hodgkin disease, ovarian cryopreservation, ovarian transplantation, heterotopic, pregnancy, live birth

Ovarian cryopreservation followed by autotransplantation is one of the several experimental strategies to preserve and restore fertility in women undergoing gonadotoxic chemotherapy. In 1999 we performed the first case of orthotopic ovarian transplantation with frozen-banked ovarian tissue, which resulted in restoration of ovarian function (1). Subsequently, we developed a heterotopic ovarian transplantation technique where the tissues were transplanted subcutaneous in the forearm (2) or lower abdomen (3), which resulted in restoration of hormonal function, oocyte recovery, and embryo generation. Since then numerous cases of orthotopic and very few heterotopic ovarian transplant cases were reported, and several of those were associated with pregnancies and live births (4–11).

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The advantages of heterotopic ovarian transplantation are the simplicity and noninvasiveness of the surgical technique, as well as the ability to closely monitor the tissue for a possibility of cancer recurrence. The disadvantages of heterotopic transplants include cosmetics, which in our experience has not been an issue, potential adverse effects to oocytes from an environment different from the pelvic intra-abdominal, and the presumed need for assisted reproduction to achieve fertility.

Here we report recurrent spontaneous pregnancies after a heterotopic ovarian transplantation to a Hodgkin lymphoma survivor who was rendered menopausal because of preconditioning chemotherapy before hematologic stem cell transplantation (HSCT). We previously reported the transplantation technique and the first and second spontaneous pregnancy from this patient; the current report focuses on the recurrent pregnancies and the long-term follow-up after that transplantation (12).

### OVARIAN TRANSPLANTATION AND LONG-TERM FOLLOW-UP

The ovarian cryopreservation and transplantation protocols were approved by the Institutional Review Board. Cancer treatment, ovarian tissue cryopreservation and heterotopic transplantation procedures, and two pregnancies that resulted in one live birth were reported previously (12). In brief, a 28-year-old Hodgkin lymphoma

survivor had premature ovarian failure immediately after HSCT and remained menopausal as confirmed by elevated serum FSH levels ranging from 46.4 to 96.6 mIU/mL and hot flashes (12). Two and a half years after HSCT, the patient requested ovarian tissue transplantation. Symptoms and signs of ovarian function and follicular growth in the graft were noted 2 months after the transplantation, and she had her first menstrual bleeding 3 years after the HSCT. She had two pregnancies on two consecutive cycles resulting in one miscarriage and one live birth at term, 13 months after the transplantation (12).

Four-month postpartum FSH and LH levels were menopausal (60.7 and 33.9 mIU/mL, respectively), indicating that the ovarian transplant ceased functioning. Six months after delivery the patient started having follicular growth in the graft in snatches but did not have a period for a year. One year after the first delivery (25 months after the transplantation) menstrual periods resumed 30 to 60 days apart. Serum FSH and LH levels on the 2nd or 3rd day of cycle ranged between 10.7 and 32.6 mIU/mL and 8.2 and 27.6 mIU/mL, respectively, giving the impression of diminished ovarian reserve. One month later, we performed ovarian stimulation using letrozole 5 mg/d in combination with recombinant FSH 75 IU (follitropin alfa injection, Gonal-F; Serono, Rockland, MA) starting on the 3rd day of her menstrual cycle (13). On day 9, two follicles were noted in the abdominal transplant (Fig. 1) and one in the in situ ovary. However, the follicle in the in situ ovary did not further develop, and the percutaneous aspiration of the SC graft resulted in the retrieval of an MII oocyte that did not fertilize. In the subsequent months, follicular activity ceased, FSH increased to 76.9 mIU/mL, and the in situ ovary remained atrophic by ultrasound examination. The patient was given oral contraceptive pills for 8 weeks and then underwent ovarian stimulation with high-dose gonadotropins (recombinant FSH 150 IU, Follistim; Organon, Roseland, NJ) + hMG 150 IU (Menopur; Ferring Pharmaceuticals, Parsippany, NJ) at a local infertility clinic with the hope that her in situ ovary might respond to ovarian stimulation. The cycle was cancelled after 7 days of stimulation because no follicles developed in the graft, and the in situ ovary remained invisible by transvaginal ultrasonography. Forty-eight hours after cancellation, the patient felt follicular growth in the graft, and 4 days later she had right-sided mittelschmerz (on

the side of the remaining ovary) and had intercourse. Pregnancy test was positive 2 weeks later. After an uneventful pregnancy, 4.5 years after transplantation, she delivered a healthy boy weighing 4,173.1 g and 54.6 cm long at term.

Six months after the delivery and after having three irregular menstrual cycles 33 to 36 days long with simultaneous follicle growth in the graft, the patient conceived spontaneously again. A transvaginal ultrasound examination 5 years and 2 months after the heterotopic ovarian transplantation confirmed an ongoing pregnancy at 6 weeks 6 days of gestation. Subsequent ultrasound scans showed a normally developing pregnancy. Because of large for gestational age fetus, the patient underwent a labor induction and delivered a girl weighing 3,263.9 g and measuring 50.8 cm 35 weeks and 5 days of gestation in April 2010 (Fig. 2).

## DISCUSSION

There are several reports of spontaneous pregnancies and live births after aggressive cancer treatment including HSCT (14–16). The duration of the return of ovarian function after HSCT varies. Therefore we cannot rule out the possibility that this patient could get pregnant even if she did not have heterotopic transplantation. However, the patient's in situ ovary appeared to be atrophic in some ultrasound examinations and was invisible in others. Ovarian follicle activity was documented by ultrasound examination in the in situ "menopausal" ovary on two occasions and was suspected by mittelschmerz-type pain on another occasion. Further, the third pregnancy occurred after she did not respond to high doses of gonadotropins and her in situ ovary was invisible by ultrasound examination.

Recent animal studies suggested the possibility of oocyte regeneration in mammals (17), but the confirmation and proof in human ovary are lacking. The work by Johnson et al. indicated that egg manufacturing could continue into adult life in mice and that germline stem cells could have originated from the bone marrow (18, 19). The same group also showed that oophorectomy completely abolished the expression of the germline stem cell markers in bone marrow in rodents. However, the replacement of estrogen and/or P did not restore the expression of those markers. The authors postulated that there might be a communication between the ovary and the bone marrow as a peripheral germline stem cell reservoir; however, this communication was not via sex hormones (19).

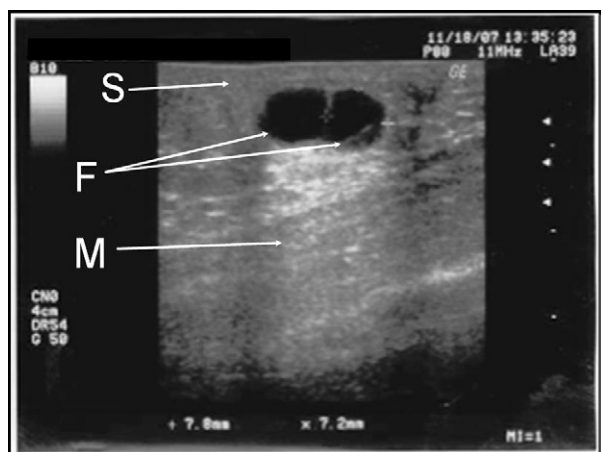
In our patient, ovarian function did not recover after HSCT. She became fertile after heterotopic ovarian tissue transplantation. If this was not coincidental, the recovery of fertility could not be linked only to HSCT; there must be an alternative mechanism.

Research in numerous nongermline stem cells indicates that the niche and microenvironment are crucial in the survival, function, and development of stem cells (20–22). We recently demonstrated that ovarian cortical pieces that did not contain developing follicles produced less estrogen in vitro if they were exposed to chemotherapeutic agents (23). Our preliminary data suggest that chemotherapy agents may damage ovarian stroma and microvessels directly (24).

We therefore hypothesize that chemotherapy treatment may not only damage ovarian follicle reserve but can also alter the ovarian niche that normally may function to attract or nurture germline stem cells for oocyte replenishment in response to an insult to the ovary. When an ovary is transplanted heterotopically, its chemotherapy-unexposed healthy ovarian niche may provide endocrine-paracrine signals to presumed reservoirs of germline stem cells such as the bone marrow, which then release these germ cell precursors to find their way to the in situ ovary by circulation.

**FIGURE 1**

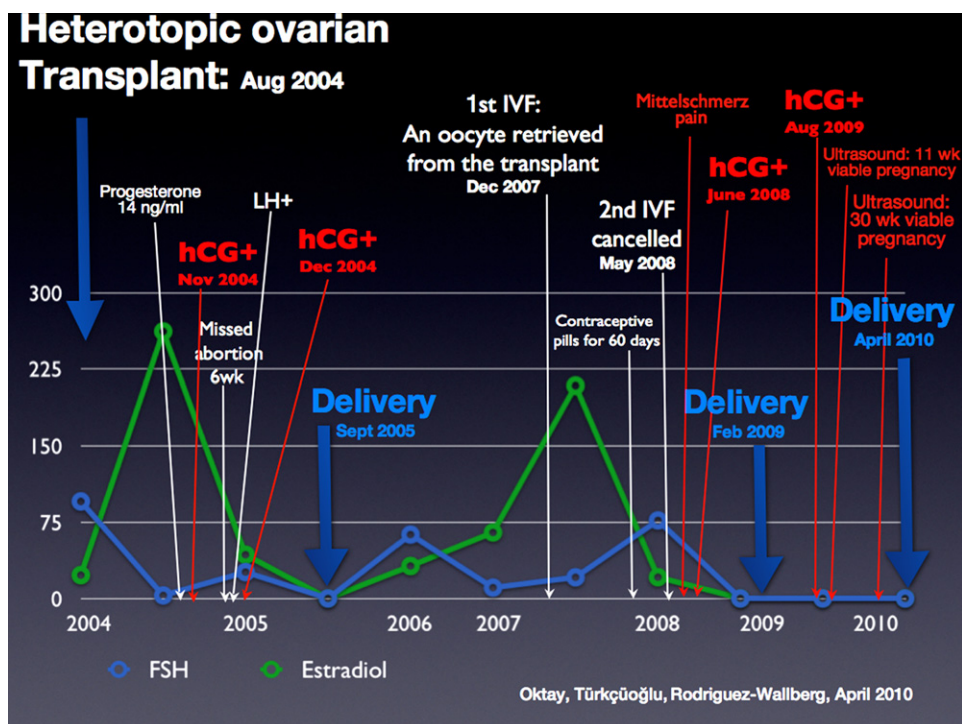
Subcutaneous follicle development after ovarian transplantation. S = skin; M = abdominal muscles; F = follicles.



Okay. Live births after SC ovary transplantation. *Fertil Steril* 2011.

**FIGURE 2**

Summary and timeline for ovarian function and recurrent pregnancies after heterotopic ovarian transplantation.



Oktay. Live births after SC ovary transplantation. *Fertil Steril* 2011.

Another possibility is that paracrine-endocrine signals from the transplanted ovary reach the damaged in situ ovary by circulation and induce generation of oocytes from the resident stem cells, which otherwise cannot be activated because of the absence of stimulatory signals from the damaged niche.

Another possible explanation for functional recovery of the in situ menopausal ovary is germ cell transfer between the transplanted and in situ ovaries. Bukovsky (25) put forward a hypothesis that instead of the periodic flow of putative bone marrow-derived germ cells to the ovaries, the ovarian germ cells periodically may enter bone marrow via the bloodstream. In parallel to that hypothesis, if ovarian germ cells residing in the transplanted ovary enter the bloodstream, they will try to find an appropriate niche for their survival, which in

our case will be the in situ ovary. The latter hypothesis assumes that chemotherapy does not damage the ovarian stem cell niche. During embryogenesis, primordial germ cells migrate from the yolk sac (which is also the origin of hematopoietic stem cells) through the dorsal mesentery of the hindgut to the genital ridges (26, 27). This migration and homing within the gonadal ridge requires integrated signals involving contact of primordial germ cells with extracellular matrix proteins and cellular substrates. It is possible that, in adult life, this rudimentary mechanism will aid the settling of circulating ovarian germ cells presumably originating from the transplanted ovary. Further laboratory and clinical research will be needed to explore these hypotheses, as well as the true origin of pregnancies after ovarian transplantations.

## REFERENCES

- Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. *N Engl J Med* 2000;342:1919.
- Oktay K, Economos K, Kan M, Rucinski J, Veeck L, Rosenwaks Z. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. *J Am Med Assoc* 2001;286:1490–3.
- Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, et al. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004;363:837–40.
- Sanchez-Serrano M, Crespo J, Mirabet V, Cobo AC, Escriba MJ, Simon C, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. *Fertil Steril* 2010;93:268.e11–3.
- Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Yemini Z, et al. Monitoring the ovaries after autotransplantation of cryopreserved ovarian tissue: endocrine studies, in vitro fertilization cycles, and live birth. *Fertil Steril* 2007;87:418.e7–15.
- Rosendahl M, Loft A, Byskov AG, Ziebe S, Schmidt KT, Andersen AN, et al. Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report. *Hum Reprod* 2006;21:2006–9.
- Ernst E, Bergholdt S, Jorgensen JS, Andersen CY. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. *Hum Reprod* 2010;25:1280–1.
- Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004;364:1405–10.
- Demeestere I, Simon P, Buxant F, Robin V, Fernandez SA, Centner J, et al. Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. *Hum Reprod* 2006;21:2010–4.
- Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod* 2008;23:2266–72.

11. Meirou D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med* 2005;353:318–21.
12. Oktay K. Spontaneous conceptions and live birth after heterotopic ovarian transplantation: is there a germline stem cell connection? *Hum Reprod* 2006;21:1345–8.
13. Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. *J Clin Oncol* 2005;23:4347–53.
14. Salooja N, Chatterjee R, McMillan AK, Kelsey SM, Newland AC, Milligan DW, et al. Successful pregnancies in women following single autotransplant for acute myeloid leukemia with a chemotherapy ablation protocol. *Bone Marrow Transplant* 1994;13:431–5.
15. Salooja N, Szydlo RM, Socie G, Rio B, Chatterjee R, Ljungman P, et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. *Lancet* 2001;358:271–6.
16. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood* 1996;87:3045–52.
17. Lee HJ, Selesniemi K, Niikura Y, Niikura T, Klein R, Dombkowski DM, et al. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. *J Clin Oncol* 2007;25:3198–204.
18. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature* 2004;428:145–50.
19. Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. *Cell* 2005;122:303–15.
20. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 2003;425:836–41.
21. Tumber T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, et al. Defining the epithelial stem cell niche in skin. *Science* 2004;303:359–63.
22. Mathur D, Bost A, Driver I, Ohlstein B. A transient niche regulates the specification of *Drosophila* intestinal stem cells. *Science* 2010;327:210–3.
23. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. *Cancer* 2007;110:2222–9.
24. Soleimani R, Heytens E, Ozkavukcu S, Lee S, Wang X, Rottiers I, et al. Impact of doxorubicin on human ovarian primordial follicles and ovarian microvasculature. *Rep Sci Sup* 2010;17:338A.
25. Bukovsky A. Can ovarian infertility be treated with bone marrow- or ovary-derived germ cells? *Reprod Biol Endocrinol* 2005;3:36.
26. Peters H. Migration of gonocytes into the mammalian gonad and their differentiation. *Philos Trans R Soc Lond B Biol Sci* 1970;259:91–101.
27. Palis J, Yoder MC. Yolk-sac hematopoiesis: the first blood cells of mouse and man. *Exp Hematol* 2001;29:927–36.